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Enantiomeric composition analysis of pranoprofen in equine plasma and urine by chiral liquid chromatography–tandem mass spectrometry in selected reaction monitoring mode

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ABSTRACT

The enantioseparation of pranoprofen after its addition in racemic form into equine plasma and urine was conducted by chiral liquid chromatography–tandem mass spectrometry in selected reaction monitoring mode. The methods for the assay of both enantiomers were linear ($r \geq 0.9943$) in the low range from 0.001 to 0.1 μg/mL and high range from 0.01 to 1.0 μg/mL with good precision (% RSD ≤ 5.6) and accuracy (% RE = −5.3 to 1.9). When racemic pranoprofen was orally administered to four horses at a single dose of 3.1 mg/kg, the median plasma concentrations of (*R*)-pranoprofen were lower than the levels of (*S*)-pranoprofen from start to finish. In contrast, the urinary level of (*R*)-pranoprofen was 2.5 fold higher than (*S*)-pranoprofen level for the first 6 h, followed by its rapid decrease down below (*S*)-pranoprofen concentration. Monitoring of the *R*/*S* ratios in equine urine may be useful for the prevention of false positive in horse doping test.

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1. Introduction

Profens constitute an important group of non-steroidal anti-inflammatory drugs which are effectively used for clinical treatments in horses [1–7]. However, they are frequently doped in horse racing to mask clinical signs of inflammation and pain in racehorses [8–13]. Hence, it has become important tasks to confirm whether positive samples are contaminated with profen standards in horse doping laboratories, to trace the administration times on positive samples during investigation and to reestablish thresholds and withdrawal times of profens for therapeutic purpose. The anti-inflammatory activities of profens are mostly due to the (*S*)-enantiomer [14]. However, they are primarily marketed as racemic mixtures. (*S*)-Profens and their antipodes differ markedly in pharmacologic effects and pharmacokinetics [15]. Hence, the accurate measurements of (*R*)- and (*S*)-profen levels in plasma and urine will come in useful for the prevention of false positive in horse doping test [16].

Among the diverse profens, ketoprofen [3,5,16], vedaprofen [4], carprofen [6], flurbiprofen [7], and fenoprofen [17] had been extensively studied for their enantioselective pharmacokinetic characteristics in the horse. Pranoprofen, 2-(5H-chromeno[2,3-b]pyridin-7-yl)propanoic acid, has been used as a safe and effective anti-inflammatory alternative for the treatment of ocular inflammation [18]. Its enantioselective metabolism has been thoroughly investigated in mice [19,20], rabbits [21,22] and beagle dogs and rats [23], but not in horses. In a recent study [16], we observed the predominance of (*S*)-ketoprofen in equine plasma and urine after intravenous administration of racemic ketoprofen by gas chromatography–mass spectrometry in selected ion monitoring mode after (*S*)-(−)-1-phenylethylamidation. The results were attributed to the unidirectional metabolic chiral inversion of (*R*)-ketoprofen occurred in the horse [3,5].

The present work was newly attempted to examine whether the differences between concentrations of (*S*)-pranoprofen and (*R*)-pranoprofen in equine plasma and urine following oral administration of racemic pranoprofen to horses can be used as an indicator suitable for the prevention of false positive in horse doping test. In this study, the chiral resolution and selective quantitation of pranoprofen enantiomers were conducted by chiral liquid chromatography–tandem mass spectrometry in selected reaction

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monitoring mode. The chemical structures of pranoprofen and mefenamic acid used as the internal standard were readily confirmed by product-ion mass spectral patterns of their respective precursor ions.

2. Materials and methods

2.1. Chemicals and reagents

Pranoprofen in racemic form and Pransus syrup®, an oral product containing racemic pranoprofen (7.5 mg/mL), were provided by Kolon Pharmaceutical, Inc. (Seoul, Korea). Enantiomerically pure (*S*)-pranoprofen (98.2%) and (*R*)-pranoprofen (97.2%) were the kind gifts from Prof. Teruko Imai (Faculty of Pharmaceutical Sciences, Kumamoto Univ., Kumamoto, Japan). Mefenamic acid was purchased from Sigma–Aldrich (St. Louis, MO, USA), and methanol, diethyl ether, hexane, ethyl acetate, toluene and dichloromethane of spectroanalyzed grade from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were of analytical-reagent grade and used as received. β-Glucuronidase/arylsufatase from Helix pomatia was provided by Roche (Indianapolis, IN, USA)

2.2. Preparation of standards, reagent and calibration biosamples

Each stock solution of racemic pranoprofen, (*S*)-pranoprofen, (*R*)-pranoprofen and mefenamic acid served as the internal standard (IS) were made up at 1 mg/mL in methanol in their free acid forms. The working stock solutions were subsequently prepared by diluting each stock solution to 20 μg/mL with methanol. All standard solutions were then stored at 4 °C. Seven calibration biosamples both for plasma and urine were freshly prepared in the following way: a 5 mL was taken from the working stock solution of racemic pranoprofen into a volumetric flask (50 mL) and evaporated into dryness under a nitrogen stream. The residue was dissolved in 10 mL of blank plasma and then further diluted to a total volume of 50 mL with blank plasma to prepare a plasma calibration sample containing each enantiomer at 1.0 μg/mL. A 15 mL of this solution was then diluted to a total volume of 50 mL with the same blank plasma to prepare a plasma calibration sample containing each enantiomer at 0.3 μg/mL. Serial dilutions with blank plasma were continued to prepare the rest plasma calibration samples containing each enantiomer at 0.1, 0.03, 0.01, 0.003 and 0.001 μg/mL, respectively. Seven urine calibration samples were prepared in the same manner as for plasma samples.

2.3. Instrumentation

LC–MS/MS analyses in selected reaction monitoring (SRM) mode were performed with an Agilent 1100 series HPLC system (Agilent Technologies, Atlanta, GA, USA) coupled to an API 2000™ tandem mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a turbo ion spray ion source operated in the positive ionization mode. Enantiomeric separations were carried out on a Chirex™ 3005 chiral stationary phase (CSP) based on (*R*)-1-naphthylglycine and 3,5-dinitrobenzoic acid amide linkage (250 × 2.0 mm i.d., Phenomenex, Torrance, CA, USA) in RP mode. The column protected by a guard column (30 × 2.0 mm i.d.) packed with the same type of CSP was maintained at 30 °C. Water–methanol (5:95) containing 0.1% of formic acid (v/v) was used as the optimum mobile phase at a flow rate of 0.2 mL/min. An aliquot (10 μL) of each sample was introduced into the LC–MS/MS system. The entire chromatographic effluent was passed into the mass spectrometer interface which was maintained at 450 °C under 5500 V. The pressures of nebulizer gas, heater gas, curtain gas and collision-activated dissociation gas (nitrogen) were 276, 552, 138 and 28 kPa, respectively. The potentials of declustер, entrance and collision cell exit were optimally set to 30, 11 and 25 V, for pranoprofen, and their corresponding values were 15, 10 and 10 V for IS. Each collision energy for pranoprofen and IS was 50 and 20 eV, respectively. Pranoprofen in unknown samples was detected in the Product-Ion SCAN (PIS) mode. In the SRM transition mode, quantitative analyses were performed with Q1 and Q3 operated in unit resolution. The precursor ion was at m/z 256 and the product ion at m/z 210 was selected for the quantitation of (*R*)-pranoprofen and (*S*)-pranoprofen. The ion at m/z 242 was the precursor ion and the product ion at m/z 224 was selectively monitored for IS. A dwell time was set to 500 ms for each selected ion.

2.4. Sample preparation for pranoprofen measurements in syrup and equine plasma

An aliquot (0.133 mL) of Pransus syrup® used for oral administration of pranoprofen in this study was dissolved in deionized water (1 L). A 2.0 mL taken from the diluted solution was adjusted to pH ≤ 2.0 with 1.0 M HCl after the addition of IS (1.0 μg). The sample was then extracted with 6 mL of a ternary solvent composed of dichloromethane, hexane and diethyl ether at a ratio of 1:1:1 (v/v/v). The extract was evaporated to dryness under a gentle stream of nitrogen gas (65 °C) and the residue was reconstituted in 0.1 mL of 0.1% formic acid in methanol for LC–MS/MS analyses. An aliquot (2 mL) of equine plasma sample was subjected to the solvent extraction in the same manner as for syrup sample.

2.5. Sample preparation for measurements of total pranoprofen in equine urine

An aliquot (1 mL) of equine urine sample was incubated (2 h at 65 °C) after the addition of β-glucuronidase/arylsulfatase (50 μL) and 1 mL of 1.0 M acetate buffer (pH 5.5). Solid-phase extraction (SPE) of pranoprofen was subsequently conducted employing GX-274 ASPEC™ (Gilson, Inc., Middleton, WI, USA) automated SPE system. Discovery® DSC-18 SPE tube (Sigma–Aldrich/Supelco, Bellefonte, PA, USA) containing 100 mg of the sorbent was preconditioned with methanol (2 mL) and water (2 mL). Each incubated urine sample spiked with IS (1.0 μg) was loaded onto the SPE column. The column was then sequentially washed with water (2 mL), 1 M acetic acid (2 mL) and hexane (2 mL), followed by blowing under a stream of nitrogen (2 min). Finally, each SPE column was eluted with 2 mL of a binary solvent mixture composed of hexane and ethyl acetate (1:1; v/v). The eluate was evaporated to dryness under a stream of nitrogen at 70 °C and the residue was reconstituted in 0.1 mL of 0.1% formic acid in methanol for LC–MS/MS analyses.

2.6. Method validation for pranoprofen measurements in equine plasma and urine

The quantitative calculation of each enantiomer was based on the peak area ratios relative to that of IS. Method validations for the measurements of pranoprofen enantiomers in plasma and urine were conducted with the seven calibration biosamples containing each pranoprofen enantiomer at different amounts (0.001, 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 μg/mL) and a constant amount of IS (0.5 μg/mL for plasma and 1.0 μg/mL for urine). An aliquot (2 mL) from plasma calibration samples and an aliquot (1 mL) from urine calibration samples were individually subjected to the sample preparation procedures as described in the preceding sections for plasma and urine, respectively. Linearity was tested using least squares regression analysis for the corrected peak area ratios against the increasing amount ratios of each enantiomer. The precision expressed as percentage of relative standard deviation (% RSD) and accuracy as percentage of relative error (% RE) were deter-

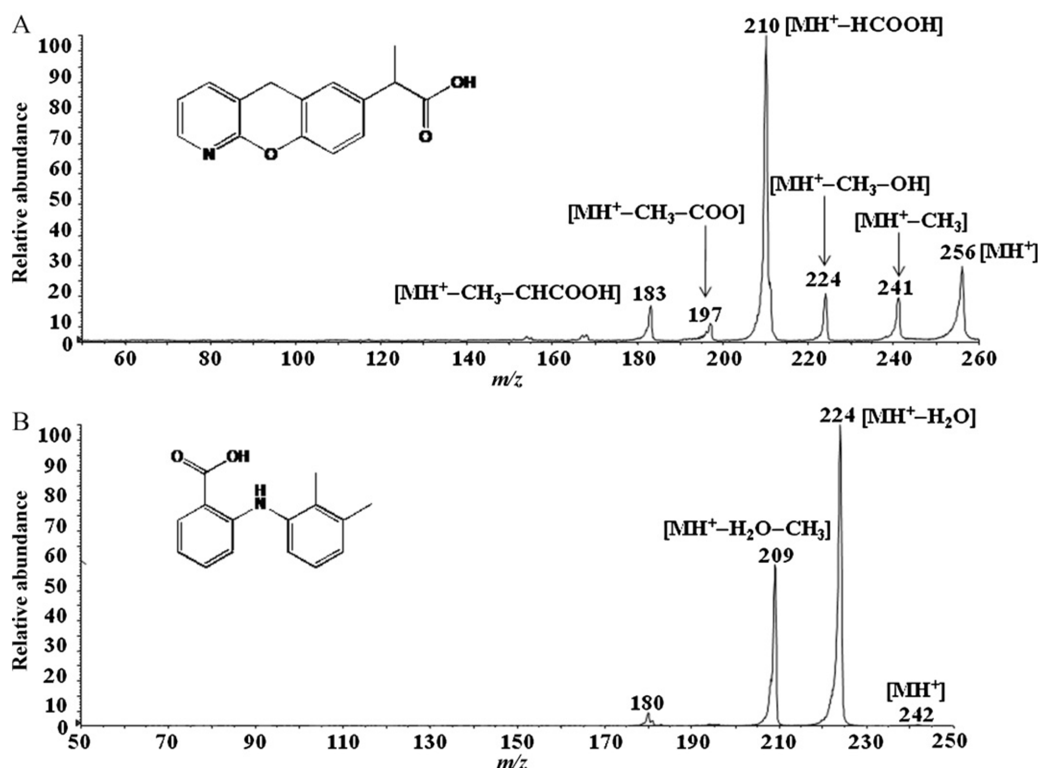

Fig. 1. Product-ion spectra of precursor ions of (A) pranoprofen and (B) mefenamic acid.

mined with two different concentrations of each enantiomer in plasma sample (0.025 and 0.25 μg/mL) and urine sample (0.03 and 0.3 μg/mL) in triplicate.

2.7. Animals, drug administration, and sample collection

Four thoroughbred mares (7–12 years, 340–403 kg) received single treatments of racemic pranoprofen at a dose of 3.1 mg/kg through oral administration. For this purpose, a small amount of feed was premixed with aliquots (140–160 mL) of Pransus syrup® (corresponding to ca. 3.1 mg/kg for the racemate). Blood samples were withdrawn into lithium heparin tubes from jugular vein at intervals (10, 20, 30, 45 and 60 min, and 1.5, 2, 3, 4, 6, 8, 12, 18, 24 and 48 h) following treatment. After centrifugation (4 °C, 1250 × g, 4 min), plasma was separated and stored at −20 °C before analysis. Urine samples separately collected at 2 and 6 h and daily for 10 days were stored at −20 °C before analysis.

Major pharmacokinetic parameters were computed by non-compartmental analysis of plasma concentration–time curve data employing WinNonlin software (Pharsight Corporation, Mountain View, CA, USA) [24].

3. Results and discussion

3.1. Product-ion spectral patterns of pranoprofen and mefenamic acid

When the MS/MS was operated in the positive ionization mode, the Q1 SCAN spectrum showed the precursor ion with much higher (over 16 times) intensity as compared to the operation in the negative ionization mode. The product-ion mass spectrum of pranoprofen (Fig. 1A) showed five product ions. The $[M+H]^+$ precursor ion at *m*/*z* 256 was very prominent with 24.8% relative abundance (RA). The product ion at *m*/*z* 210 constituted the base peak ion which was formed by the removal of HCOOH through the preferential benzylic cleavage from its precursor ion. The next prominent ion at *m*/*z* 224 (15.9% RA) was formed by the consecutive losses of CH_3 and OH from the precursor ion but the loss of CH_3 yielded the ion at *m*/*z* 241 (14.5% RA). The losses of COO and CHCOOH from the ion at *m*/*z* 241 formed less abundant ions at *m*/*z* 197 (6.1% RA) and at *m*/*z* 183 (12% RA), respectively. The SRM transition of *m*/*z* 256 to 210 was selected as the quantitative ion to be monitored for the measurement of pranoprofen enantiomers. In contrast, the mass spectrum of mefenamic acid used as IS (Fig. 1B) showed two major product ions at *m*/*z* 224 and *m*/*z* 209 with a minor ion at *m*/*z* 180. The $[M+H]^+$ precursor ion at *m*/*z* 242 was almost unobservable. The most prominent ion at *m*/*z* 224 was formed by the selective removal of H_2O from its precursor ion. The next abundant ion at *m*/*z* 209 (53.6% RA) was produced by the simultaneous elimination of H_2O and CH_3 from the precursor ion. No peak ion formed by the removal of HCOOH from the precursor ion was detected, which was the most prominent in the pranoprofen mass spectrum. The SRM transition of *m*/*z* 242 to 224 was chosen as the quantitative ion for IS.

3.2. Enantiomeric separation of pranoprofen

The Chirex CSP column used in this study required to modify methanol mobile phase with 5% water containing 0.1% of formic acid (v/v) to achieve complete enantiomeric resolution of racemic pranoprofen within 13 min. However, the column was stable for several months during the whole experiments. Mefenamic acid served as IS eluted earlier with a good peak shape. When the HPLC system was linked to the highly selective and sensitive MS/MS in the SRM mode, very selective monitoring for each enantiomer present in equine plasma and urine samples was achieved without any undesirable interference. A typical LC–MS chromatogram in SRM mode showed a very clean and simple enantiomeric profile of an equine urine sample spiked with known amount of racemic pranoprofen (Fig. 2). The SRM detection of pranoprofen

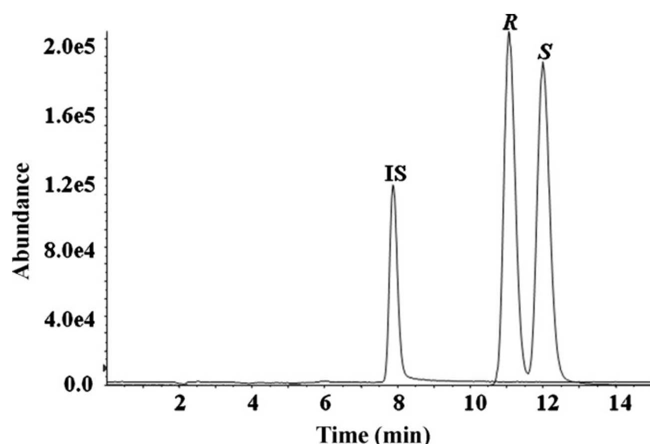

Fig. 2. LC–MS chromatogram in SRM mode of (*R*)- and (*S*)-pranoprofen enantiomers, and mefenamic acid (IS) spiked into equine urine (0.6 μg/mL).

enantiomers effectively eliminated the interfering problem caused by co-extraction of multiple endogenous organic acids present at much higher concentrations in urine specimens.

3.3. Method validation for the measurement of pranoprofen enantiomers in equine plasma and urine

The measurements of each pranoprofen enantiomer from the plasma calibration samples showed good linearity ($r \geq 0.9978$) in the low calibration range (0.001, 0.003, 0.01, 0.03 and 0.1 μg/mL), and high calibration range (0.01, 0.03, 0.1, 0.3 and 1.0 μg/mL) (Table 1). For the urine specimens, the linearity was acceptable in the lower calibration range, while the measurements showed excellent linearity ($r \geq 0.9991$) in the higher range. The overall linearity proved suitability of the present method for quantitative assay of pranoprofen enantiomers in unknown samples. When the present method was tested with quality control samples at two concentrations for each enantiomer, the precision (% RSD) and accuracy (% RE) in plasma were varied from 2.3 to 5.6 and from −5.3 to 1.9, respectively. From urine samples the precision (% RSD) and accuracy (% RE) ranged from 2.5 to 3.7 and from −3.5 to −1.2, respectively. These results indicated that the concentration of each enantiomer present in plasma and urine can be determined with good precision and accuracy. No changes in racemic composition were observed during this validation study, indicating that nearly no racemization was occurred.

When the present method was applied to Pransus syrup after dilution to contain racemic pranoprofen at 1 μg/mL in triplicate, the contents of (*R*)-pranoprofen and (*S*)-pranoprofen were 0.485 ± 0.012 μg/mL and 0.493 ± 0.014 μg/mL, respectively. This result showed a racemic composition of 50:50.

3.4. Enantiomeric composition in equine plasma and urine following oral administration of racemic pranoprofen

The four thoroughbred horses examined in this study differed in age (7–14 years) and weight (340–403 kg). When the levels in plasma and urine were monitored following a single oral administration of racemic pranoprofen at a dose of 3.1 mg/kg, large variations in the concentration of each enantiomer were observed from horse to horse. However, the overall elimination and excretion rates for both enantiomers were all comparable. Pranoprofen displayed enantioselective pharmacokinetics: median plasma concentrations of the (*R*)-enantiomer were lower than those of (*S*)-pranoprofen from start to finish, as indicated by median area under the curve ($AUC_{0-\infty}$) values for (*R*)- and (*S*)-enantiomers of 22.3 and 103.6 μg h/mL (Table 2). The maximum median concentration (C_{max}) values at t_{max} were 3.3 μg/mL at 2.0 h and 5.7 μg/mL at 2.50 h, respectively. The plasma concentration of (*R*)-pranoprofen was more rapidly decreased compared with (*S*)-pranoprofen concentration (Fig. 3A). These results were consistent with the previous reports on enantiomeric composition of pranoprofen in plasma of beagle dogs after oral administration of racemic pranoprofen [23]. No further alterations in the plasma *R*/*S* ratios were observed after 48 h. The urinary levels of

Table 1
Method validation for the measurement of pranoprofen enantiomers from equine plasma and urine.

Sample	Enantiomer	Calibration range (μg/mL)[a]	r[b]	Concentration tested (μg/mL)	Precision (% RSD)[c]	Accuracy (% RE)[d]
Plasma	(*R*)-Pranoprofen	0.001–0.1	0.9995	0.025	5.6	1.9
		0.01–1.0	0.9978	0.25	2.3	−4.6
	(*S*)-Pranoprofen	0.001–0.1	0.9992	0.025	2.5	1.9
		0.01–1.0	0.9978	0.25	4.1	−5.3
Urine	(*R*)-Pranoprofen	0.001–0.1	0.9944	0.03	3.3	−1.8
		0.01–1.0	0.9991	0.3	2.6	−2.9
	(*S*)-Pranoprofen	0.001–0.1	0.9943	0.03	3.7	−1.2
		0.01–1.0	0.9992	0.3	2.5	−3.5

All measurements were made in triplicate.
[a] First calibration range: 0.001, 0.003, 0.01, 0.03, and 0.1 μg/mL, and second calibration range: 0.01, 0.03, 0.1, 0.3 and 1.0 μg/mL.
[b] Correlation coefficient.
[c] Relative standard deviation.
[d] Relative error: {(measured mean value – nominal value)/nominal value} × 100.

Table 2
Pharmacokinetic parameters of (*R*)- and (*S*)-pranoprofen enantiomers in equine plasma after a single administration of racemic pranoprofen.

Parameters	(*R*)-Pranoprofen		(*S*)-Pranoprofen	
	Median	Range	Median	Range
t_{max} (h)	2.0	0.0	2.5	1.0
C_{max} (μg/mL)	3.4	1.8	6.0	3.6
$t_{\lambda_{1/2}}$ (h)	7.1	5.8	14.0	18.4
$AUC_{0-\infty}$ (h μg/mL)	22.3	16.7	103.6	108.2

Pranoprofen in racemic mixture was orally administered to four horses at a dose of 3.1 mg/kg.

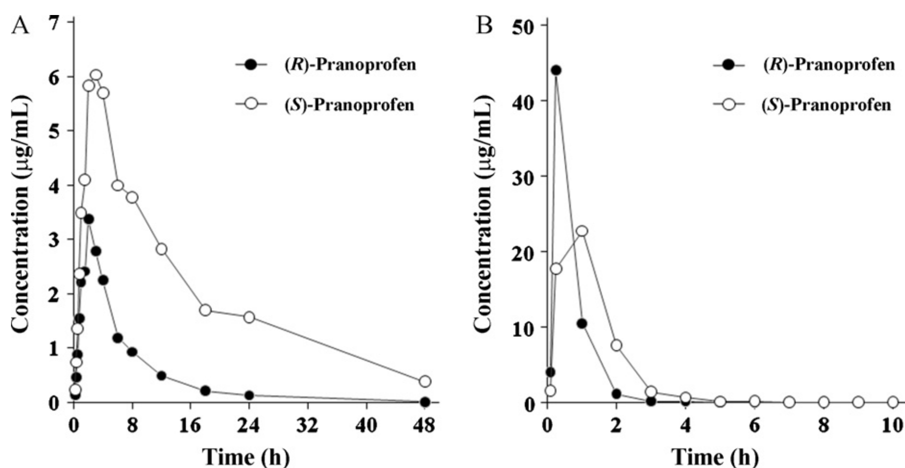

Fig. 3. Changes in median concentrations of (*R*)-pranoprofen and (*S*)-pranoprofen with time in (A) plasma and (B) urine after oral administration of racemic pranoprofen to four horses at a dosage of 3.1 mg/kg.

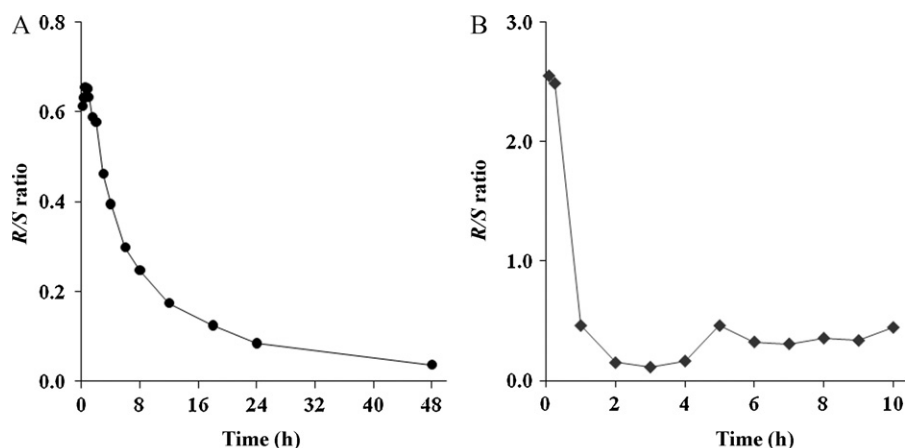

Fig. 4. Changes in ratios of median concentrations of (*R*)-pranoprofen to (*S*)-pranoprofen with time in (A) plasma and (B) urine after oral administration of racemic pranoprofen to four horses at a dosage of 3.1 mg/kg.

(*R*)-pranoprofen were 2.5 fold higher than (*S*)-pranoprofen levels for 6 h and thereafter, its levels rapidly decreased below the (*S*)-pranoprofen concentrations (Fig. 3B). This predominance of (*R*)-enantiomer in urine for the first 6 h can be explained by its enantioselective excretion. Further alterations in the urinary *R*/*S* ratios were not noticed after 10 days. In course of repetitive testing, no appreciable changes in enantiomeric composition of each plasma and urine were observed, suggesting no occurrence of racemization.

When the alteration in ratios of median concentrations of (*R*)-pranoprofen to (*S*)-pranoprofen was plotted against time, enantiomeric compositional changes were more distinctively demonstrated (Fig. 4). The plasma *R*/*S* ratios decreased down from 0.6 to 0.04 in parabolic shape (Fig. 4A). However, the urinary *R*/*S* ratios were 2.5 for 6 h and then steeply went down less than unity at 24 h and then stayed below 0.5 (Fig. 4B). The rapid change in the urinary *R*/*S* ratios suggested that monitoring of the *R*/*S* ratios in noninvasive urine will come in useful for the prevention of false positive in horse doping test.

4. Conclusions

The present chiral LC–MS/MS method in SRM mode provided selective quantitation of each pranoprofen enantiomer in equine plasma and urine with good precision and accuracy. The method is expected to be useful to confirm whether positive sample is contaminated with reference pranoprofen and to trace administration time of positive samples during investigation and to reestablish thresholds and withdrawal times of pranoprofen for therapeutic purpose in racehorses.

References

[1] W.E. Jones, J. Equine Vet. Sci. 12 (1992) 265.
[2] F.T. Delbeke, J. Landuyt, M. Debackere, J. Pharmaceut. Biomed. Anal. 13 (1995) 1041.
[3] M.F. Landoni, P. Lees, J. Vet. Pharmacol. Therap. 19 (1996) 466.
[4] P. Lees, S.A. May, M. Hoeijmakers, A. Coert, P.V. Rens, J. Vet. Pharmacol. Therap. 22 (1999) 96.
[5] C.R. Verde, M.I. Simpson, A. Frigoli, M.F. Landoni, J. Vet. Pharmacol. Therap. 24 (2001) 179.
[6] P. Lees, F.S. Aliabadi, M.F. Landoni, J. Vet. Pharmacol. Therap. 25 (2002) 433.
[7] A.L. Soracel, O. Tapia, J. Garcia, J. Vet. Pharmacol. Therap. 28 (2005) 65.
[8] H.H. Maurer, J. Chromatogr. B 733 (1999) 3.
[9] C. Tsitsimpikou, M-H.E. Spyridaki, I. Georgoulakis, D. Kouretas, M. Konstantinidou, C.G. Georgakopoulos, Talanta 55 (2001) 1173.
[10] A. Takeda, H. Tanaka, T. Shinohara, I. Ohtake, J. Chromatogr. B 758 (2001) 235.
[11] M.C. Dumasia, A. Ginn, W. Hyde, J. Peterson, E. Houghton, J. Chromatogr. B 788 (2003) 297.
[12] N.H. Yu, E.N.M. Ho, F.P.W. Tang, T.S.M. Wan, A.S.Y. Wong, J. Chromatogr. A 1189 (2008) 426.
[13] H. Nicolas-Frey, Annual Report on Prohibited Substances by FNCF, France, Racing Medication & Testing Consortium, USA, 2008, p. 5.
[14] M.F. Landoni, A. Soraci, Curr. Drug Metab. 2 (2001) 37.

[15] P. Lees, M.F. Landoni, J. Giraudel, P.L. Toutain, J. Vet. Pharmacol. Therap. 27 (2004) 479.
[16] J. Yu, G. Lee, Y. Jeong, Y.H. Ahn, M.B. Hu, S.J. Kim, K.R. Kim, M.J. Paik, Chromatographia 69 (2009) 59.
[17] A. Soraci, P. Jaussaud, E. Benoit, P. Delatour, Vet. Res. 27 (1996) 13.
[18] I. Akyol-Salman, D. Leçe-Sertöz, O. Baykal, J. Ocular Pharmacol. Therap. 23 (2007) 280.
[19] N. Arima, Y. Kato, J. Pharmacobiodyn. 13 (1990) 719.
[20] N. Arima, J. Pharmacobiodyn. 13 (1990) 733.
[21] T. Nomura, T. Imai, M. Otagiri, Biol. Pharm. Bull. 16 (1993) 298.
[22] T. Imai, N. Nomura, M. Otagiri, Chirality 15 (2003) 318.
[23] T. Imai, T. Nomura, T.M. Aso, M. Otagiri, Chirality 15 (2003) 312.
[24] WinNonlin™ Users Guide Ver. 3.0, Pharsight Corp., Mountain View, CA, USA, 1998–1999.